



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Douglas F. Covey  
Serial No. 10/008,567  
Filed November 5, 2001  
Confirmation No. 6682  
Examiner Evelyn Mei Huang

Art Unit 1625

For CYTOPROTECTIVE POLYCYCLIC COMPOUNDS

September 5, 2003

**DECLARATION OF DOUGLAS F. COVEY**

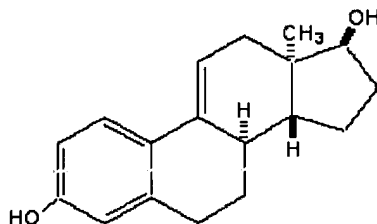
TO THE ASSISTANT COMMISSIONER FOR PATENTS,  
Washington, D.C. 20231

I, Douglas F. Covey declare as follows:

1. I am a professor employed by Washington University of St. Louis, located in the State of Missouri.
2. I am the inventor of the subject matter claimed in the above-identified U.S. Patent application, Serial No. 10/008,567.
3. I conceived and reduced to practice the invention of claim 36, as presented in Amendment B submitted herewith, prior to October 19, 2000.
4. All work referred to herein was carried out in the United States.
5. Evidence of my conception and reduction to practice of a compound having cytoprotective activity, as described by claim 36 of Amendment B submitted herewith, is attached hereto as Exhibits A, B and C. On information and belief, Exhibit A is a true and

11-2  
VcB  
9-17-3

correct copy of pages of a laboratory notebook (with dates deleted) maintained by Zu Yun Cai who, at the time the work described in these pages was performed, was working under my direction and supervision. The work described on these pages was carried out prior to October 19, 2000. Exhibit A identifies and describes the preparation of the following cytoprotective compound:



Additionally, on information and belief, Exhibits B and C are true and correct copies of NMR and Ultraviolet spectra, respectively, of the above-referenced compound (with dates deleted) maintained by Zu Yun Cai who, at the time these analyses were performed and the spectra were generated, was working under my direction and supervision. These analyses were performed and the spectra were generated prior to October 19, 2000.

Accordingly, Exhibits A, B and C evidence my conception and reduction to practice of the cytoprotective compounds of claim 36, as amended, prior to October 19, 2000.

6. I declare that all statements made herein of my knowledge are true; and further that these statements were made with the knowledge that willfully making false statements is punishable by fine, imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

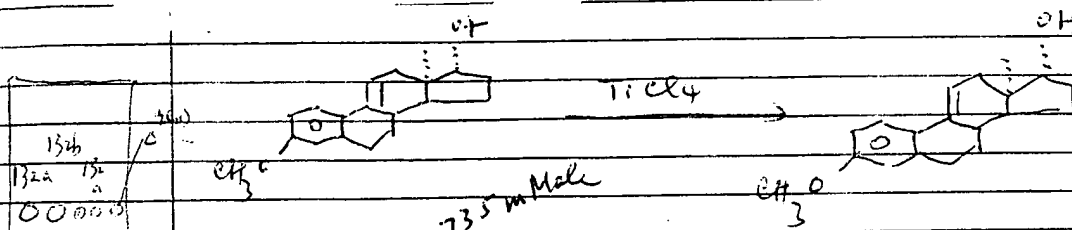
Sept. 5, 2003  
Date

Douglas F. Covey  
Douglas F. Covey

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PROJECT NAME \_\_\_\_\_

NOTEBOOK NO. \_\_\_\_\_



0.25 g (0.735 mmole) separate from  $\Delta^5$  (0.75 mmole)

50 mg, 0.15 g 201324, + 0.05 g 201324 b)

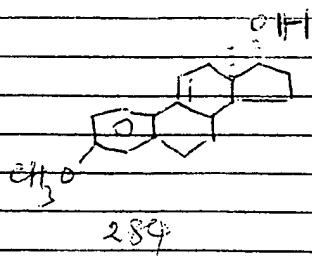
+ 10 ml anhydrous  $\text{CH}_2\text{Cl}_2$ , cool to  $0^\circ \rightarrow -10^\circ$  (outside rack) + 0.5 ml  $\text{TiCl}_4$  quickly. The reaction mixture became dark brown. Stir to end total time 20 min. Temp (outside)  $-0^\circ \rightarrow -10^\circ$ . Take TLC. The reaction is done. Then at  $0 \rightarrow 10^\circ$  add 2 ml  $\text{H}_2\text{O}$ . The dark brown color fades to colorless. Then extract  $\times$  3 times, several times. Washed  $\times$  3 times until the aqueous appear almost neutral. dried  $\times$  3 times. Remove the solvent after got crude 0.24. Run NMR 20138 OK. After work up. Take TLC as 2

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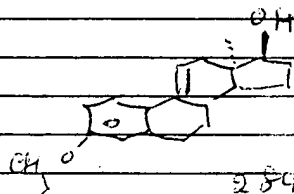
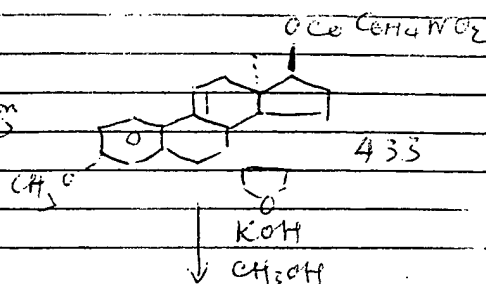
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DATE \_\_\_\_\_ 19  
DATE \_\_\_\_\_ 19

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Mitsunobu reaction



0.24 gm (0.845 m Mole)  
ST ~~0.25~~ gm (~~0.735~~ m Mole)

CH<sub>3</sub>COON = N COONH<sub>3</sub> 2.5 ~~1.84~~ 130 320 mg  
0.735 x 1.3 = ~~0.956~~ m Mole (x 174) = 166.32 g

NO<sub>2</sub> C<sub>6</sub>H<sub>4</sub> COOH 0.735 x 2.5 = ~~1.84~~ (x 167) = 306.86

Ph<sub>3</sub>P 0.735 x 1.3 = ~~0.956~~ (x 262.29) = 250.62 + 81.95 mg

ST 0.24 gm + 7 ml anhydrous toluene + NO<sub>2</sub> C<sub>6</sub>H<sub>4</sub> COOH 0.308 g

+ Ph<sub>3</sub>P 0.49 gm + ~~2.5 gm~~ Dead 0.45 g heat outside bath.

80-90°C Start 11:00 AM. — 5:00 PM. (Stop)

Take TLC

Remove the solvent as much as possible, then almost dry.  
Then Run flash chromatography 7%, 10%, 15%, 30%, 50%  
compd came down from 10%

Got 150 mg not very accurate yellow solid NMR 2013.90K

$$\frac{240}{284} \times 433 = 365.9$$

$$\frac{150}{365.9} = 41\%$$

8-10-19

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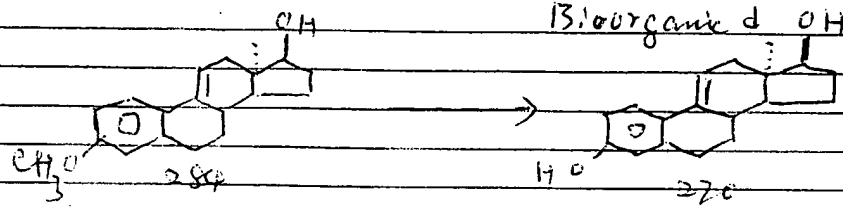
19

DATE \_\_\_\_\_

19

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Ref. J. Org. Chem. 1987, 52, 4235-4238  
 J. Biol. Chem. 187, 1950, 557, 562, 563  
 Bioorganic & Medicinal Chemistry Letter Vol. 1  
 1995



To Under N<sub>2</sub> 1.5M DIBAL 1.5ml DIBAL = 2.25mMals was added  
 60mg (0.21mMals) in 3ml toluene solution ST.V & heat  
 outside bath 120°C 11:30 - overnight.

Temperature keep 125°C (outside bath)

Take TLC, its done, add ice to the bottle,  
 acidified 3N 3ml to pH 4, extract 3x EtOAc  
 several times washed 3x mine dried 3x Na<sub>2</sub>SO<sub>4</sub>  
 to get 70mg.

Flash chromatography using 10% EtOAc in hexane 15% 30%  
 got 60mg. which crystallization from acetone & hexane  
 got 30mg 20141 mp 239-241°C

determination of [α]<sub>D</sub>

$$\frac{60}{289} \times 270 = 57. \quad \frac{40}{57} = 70\%$$

wt.	-0.348	dioxane
12.0665	-0.347	0.000
12.0612	0.348	0.000
	0.349	
0.0053 in 2ml dioxane	0.348	UV. wt wt
	0.347	11.8898
1ml = 0.00265	0.347	11.8882
	0.348	0.0016 = 1.6mg
	0.346	λ <sub>max</sub> 263.0 (CH <sub>3</sub> OH)
[α] <sub>D</sub> <sup>25</sup> = -0.348	0.347	1.6mg Abs. 1.7901
0.00265	0.349	
(C=0.265)	0.349	1.6mg in 5ml CH <sub>3</sub> OH
	0.347	take 0.5ml dilute to 5ml

average = 0.348

Send Dr Carey 25mg

$$\bar{c} = \frac{1.7901}{0.00016 \times 200} = 15106.329$$

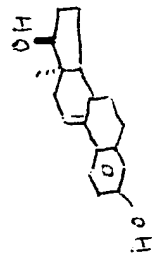
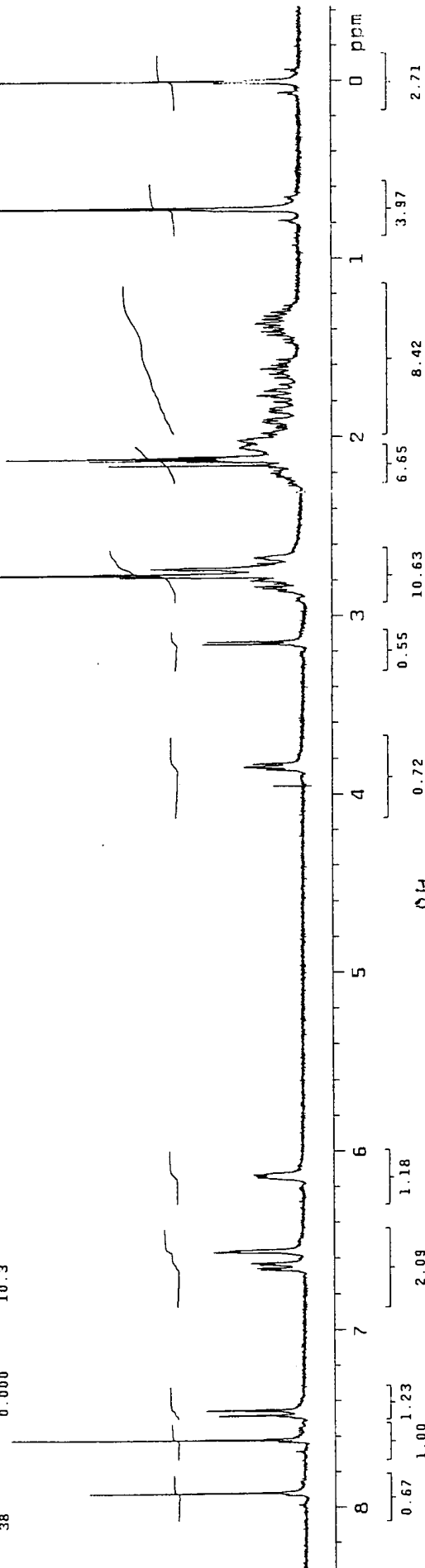
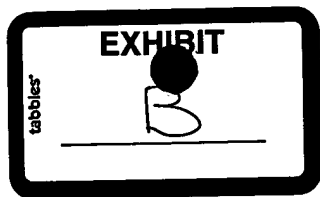
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20141  
 20141  
 327  
 stored in hexane  
 run 3 times

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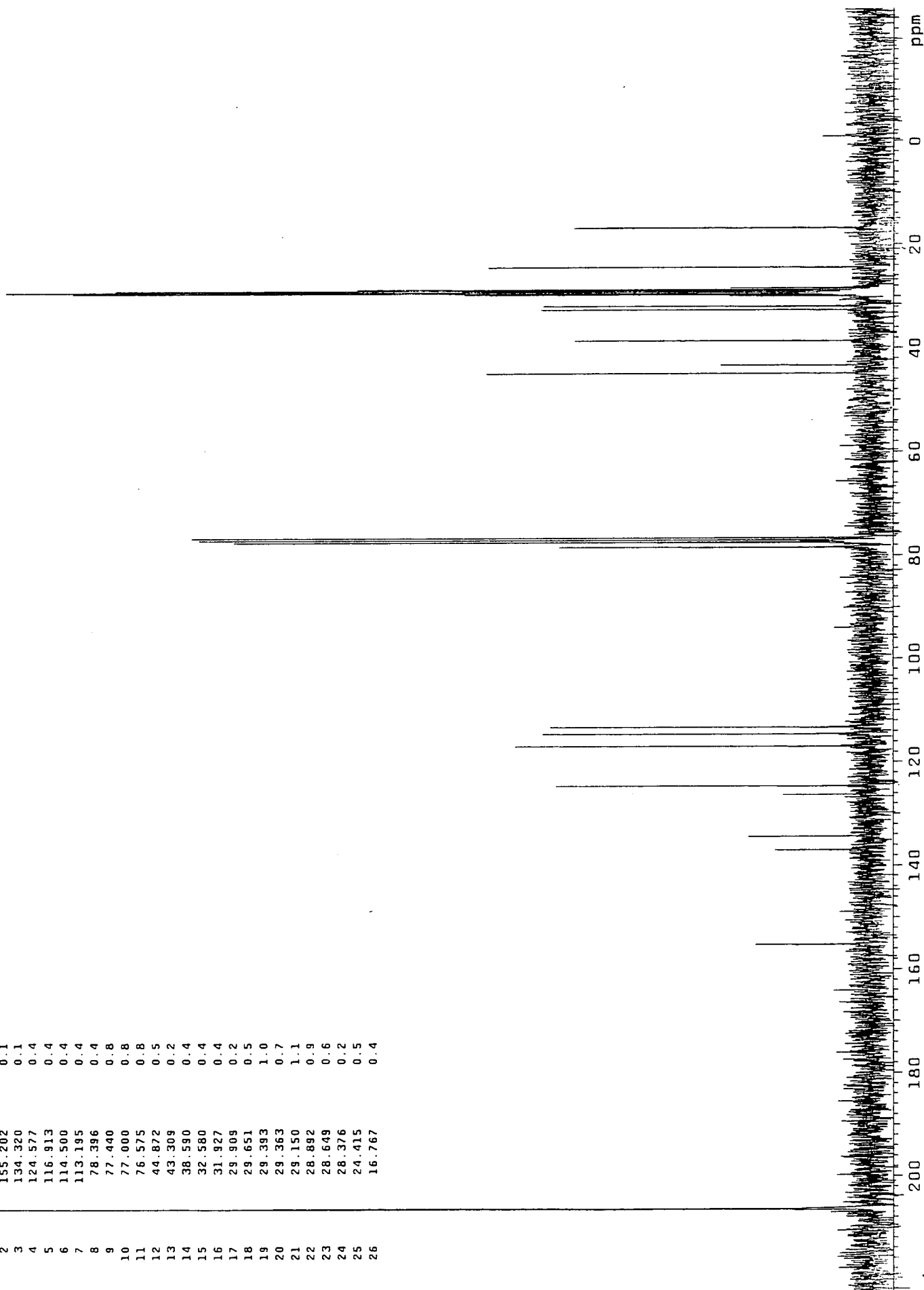
INDEX	FREQUENCY (PPM)	HEIGHT
1	7.920	3.5
2	7.620	4.8
3	7.482	1.4
4	7.453	1.6
5	6.665	0.7
6	6.656	0.8
7	6.636	0.7
8	6.528	0.9
9	6.566	1.5
10	6.556	1.2
11	6.150	0.7
12	6.140	0.8
13	6.132	0.8
14	3.845	0.9
15	3.827	0.8
16	3.158	1.6
17	3.144	1.5
18	2.835	0.8
19	2.801	0.8
20	2.781	3.0
21	2.770	25.9
22	2.734	2.5
23	2.703	0.7
24	2.670	0.8
25	2.158	3.2
26	2.136	1.9
27	2.128	3.5
28	2.121	4.9
29	2.114	3.5
30	2.106	2.0
31	2.059	1.0
32	2.036	0.9
33	2.018	1.0
34	1.770	0.7
35	1.368	0.7
36	0.719	8.3
37	0.011	1.4
38	0.000	10.3



ZYC-12

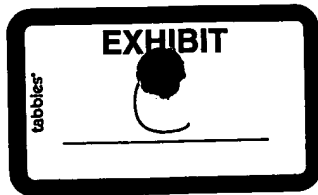
$CDCl_3: CD_3COCD_3 = 2:1$

INDEX	FREQUENCY (PPM)	HEIGHT
1	206.345	1.3
2	155.202	0.1
3	134.320	0.1
4	124.577	0.4
5	116.913	0.4
6	114.500	0.4
7	113.195	0.4
8	78.396	0.4
9	77.440	0.8
10	77.000	0.8
11	76.575	0.8
12	44.872	0.5
13	43.309	0.2
14	38.590	0.4
15	32.580	0.4
16	31.927	0.4
17	29.909	0.2
18	29.651	0.5
19	29.393	1.0
20	29.363	0.7
21	29.150	1.1
22	28.892	0.9
23	28.649	0.6
24	28.376	0.2
25	24.415	0.5
26	16.767	0.4



ZYC-12

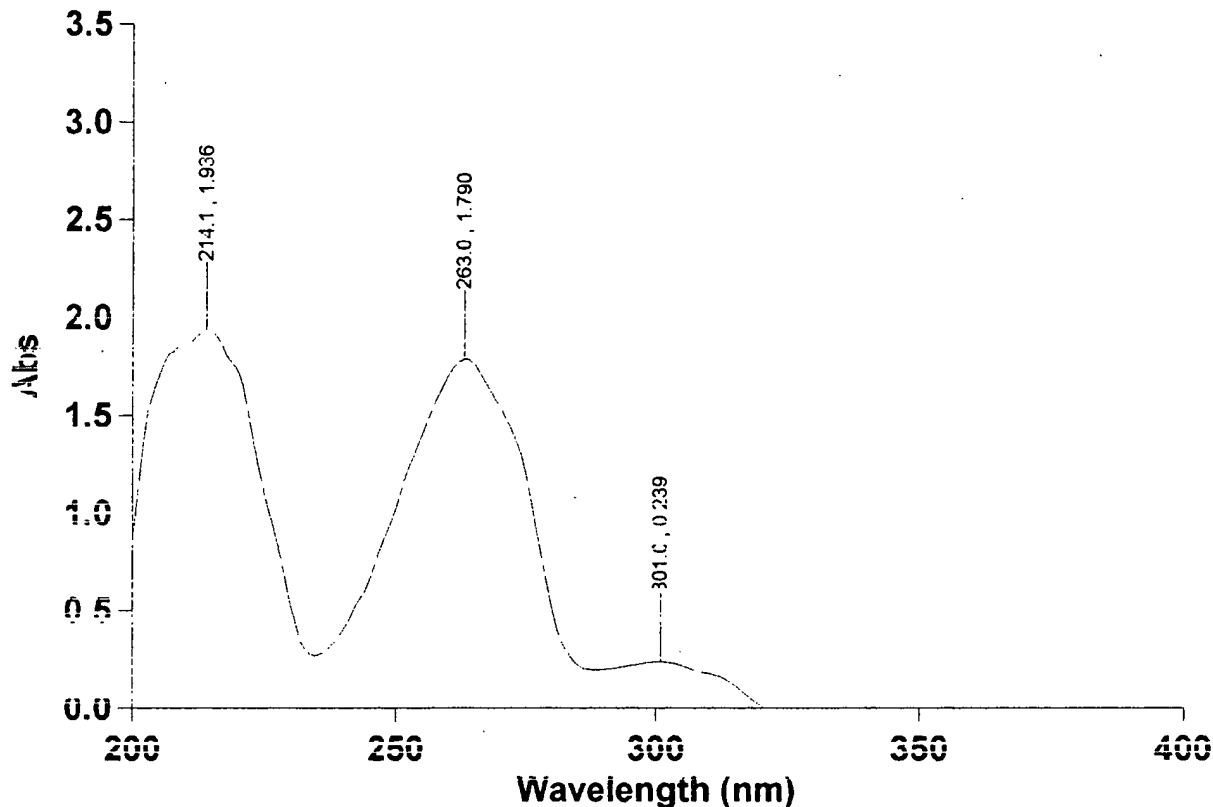
CO2: CD3COOD, 2:1



Dr Garland Marshall's LAB  
Instrument Serial Number EL98103244

1.6mg in 5ml ethanol  
Take 0.5ml dilute to 5ml.

$$\epsilon = \frac{1.7901}{\frac{0.00016 \times 200}{270}} = 15106.329$$



## Scan Analysis Report

Report Time :  
Batch:  
Software version: 02.00(25)  
Operator: guest.

Sample Name: 20141

Collection Time

2YC-12

Peak Table  
Peak Style  
Peak Threshold  
Range

Peaks  
0.0100  
400.0nm to 199.5nm

Wavelength (nm)	Abs
301.0	0.2389
263.0	1.7901
214.1	1.9359

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